FOOD BIOTECHNOLOGY

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Methods and Applications of Molecular Cloning

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INTRODUCTION

The development of molecular cloning methods (the isolation of specific DNA fragments from complex genomes and their multiplication and expression in bacterial cells) probably represents the major biological breakthrough of the decade. Not only has DNA cloning led to totally new approaches in most fields of biological research but also it promises to profoundly affect the future of mankind by its applications. Several compounds of medical and industrial importance have already been produced and are currently being marketed at competitive prices.

Among the most significant scientific achievements resulting from DNA cloning are (1) the isolation and characterization of functional genes from both prokaryotes and eukaryotes, which led to the discovery of intervening sequences that separate coding sequences of eukaryotic genes, and (2) the demonstration that the genomic organization in eukaryotic cells is not static but, rather, that genome rearrangements occur frequently. Basically, the cloning of DNA involves the in vitro joining of DNA molecules (passenger DNA to vector DNA) and the propagation of the resulting hybrid molecule into suitable host cells to obtain a clone, that is, a colony of identical cells derived from a single parental cell. The steps leading to the isolation of such clones constitute what is called molecular cloning (recombinant DNA technology). The rapid development of molecular cloning techniques resulted from progress made in several fields and has led to (1) the characterization and purification of a wide collection of enzymes that permit the modification and specific cleavage of nucleic acids, (2) the construction of cloning vehicles or vectors

derived from either bacterial plasmids, bacteriophages, or animal viruses, (3) the development of techniques that allow the efficient introduction of large pieces of DNA into bacterial and eukaryotic cells, and (4) the development of techniques to rapidly screen transformants for their DNA content or expression of cloned sequences. To put all this into perspective, this chapter will present the basic experimental methods of recombinant DNA (rDNA) techniques and their limitations, possible applications to the food industry, and future trends. An understanding of the methods and applications of recombinant DNA technology requires familiarity with the structure of genes and gene products, the nature of the genetic code, the differences in how proteins are synthesized in prokaryotes and eukaryotes, and the approaches for developing new specialized organisms. The biochemical reactions in bacteria by which genetic information is stored, transferred, and converted into proteins are reviewed in Figures 1–3.

BASIC RECOMBINANT DNA TECHNOLOGIES

Progress in genetic engineering has been highly dependent upon the development of new methods, which have been used to establish new conceptual advances in biology. Recombinant DNA technology involves in vitro DNA recombination and the transfer of rDNA into a suitable foreign host. (For detailed methods in rDNA technology, the manual on molecular cloning by Maniatis et al., 1982, is recommended.) This process can be divided into five different stages: (1) gene selection, (2) DNA isolation, fragmentation, and purification to permit cloning of the gene of interest into a vector, (3) a suitable choice of vector, (4) gene insertion into the vector and introduction of rDNA into the host, and (5) the screening and selection of clones. Figure 4 depicts the fundamental steps in rDNA technology.

Gene Selection

The desired final product will determine the source of the genes. Although the genetic code is universal, the appreciable differences—such as introns and eukaryotic transcriptional controls—between prokaryotic (bacterial) and eukaryotic (animal, plant, and fungal) genes must be evaluated to ensure subsequent expression of the desired traits.

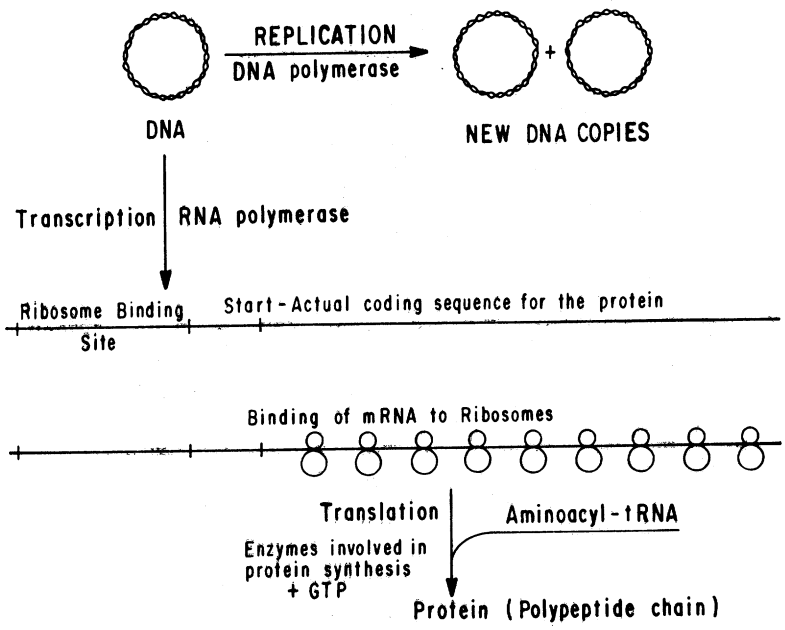

Fig. 1. The storage and conversion of genetic information is shown schematically in Figures 1 and 2. Both the *Escherichia coli* chromosome and plasmids useful in recombinant DNA methods are closed, circular, double-stranded DNA molecules. The chromosomal DNA is replicated by the enzyme DNA polymerase before cell division. Thus, genetically, both daughter cells are identically endowed (Davidson, 1969). Plasmid DNA, however, replicates independently of the chromosome (Sherratt, 1974; Helinski, 1976); one plasmid inserted into an *E. coli* may generate a hundred or more copies of itself within the cell. Expression begins with transcription of a section of DNA by the action of DNA-dependent RNA polymerase into messenger RNA (mRNA) molecules, which are single-stranded polyribonucleotides that mediate the conversion of DNA into protein (Davidson, 1969; Zillig et al., 1970; Watson, 1976). Ribosomes bind the mRNA molecules and, with these as templates, link amino acids to form proteins according to the genetic code (see Fig. 3) (Watson, 1976; Lewin, 1974; Nirenberg et al., 1966). Amino acids do not attach directly to mRNA templates. First they combine with transfer RNA (tRNA) molecules to form "activated" aminoacyl-tRNA (AA-tRNA) by a specific activating enzyme (aminoacyl synthetase) for the accurate reading of the genetic code. DNA polymerase, RNA polymerase, aminoacyl synthetase, and ribosomes owe their great fidelity of translation to the specific interactions of nucleic acids known as sequence complementarity (see Fig. 2).

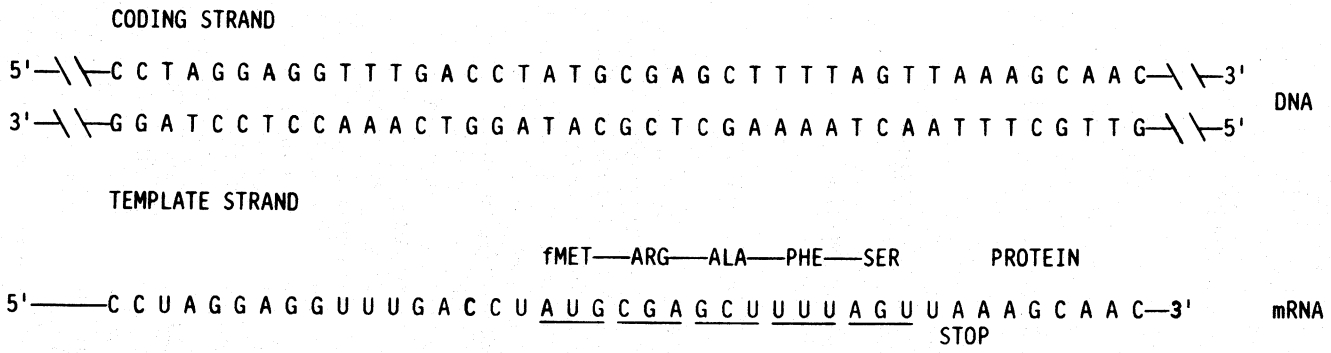

Fig. 2. Transcription and translation of genetic information is stored in the structure of double-stranded DNA. A DNA is a chain of nucleotides, each consisting of a deoxyribose sugar ring, a phosphate group, and four organic bases: adenine (A), guanine (G), thymine (T), and cytosine (C). The sugars and phosphates constitute the backbone of the strand, and paired bases linked by hydrogen bonds connect two strands. The bases linked by hydrogen bonds connect two strands. The bases are complementary: A is always opposite to T and G is always opposite to C. The genetic information depends on sequence complementarity. Expression begins when mRNA is transcribed by RNA polymerase using the lower DNA strand as a template and inserts A, C, G, and uracil (U) (chemically analogous to T) monomers according to the same complementarity rules to generate a sequence identical in biological information to the upper DNA strand. At the ribosome the complementary interaction between the triplet codon in the mRNA and the anticodon of the appropriate aminoacyl-tRNA determines which amino acid is inserted next to the growing polypeptide chain. The example here shows initiation of protein synthesis at a "start" codon (methionine codon AUG) and termination of synthesis at UAA, which is one of three possible "stop codons" in the genetic code.

THE GENETIC CODE

1ST→ 2ND	U	C	A	G	←3rd
U	PHE	SER	TYR	CYS	U
	PHE	SER	TYR	CYS	C
	LEU	SER	OCHRE	?	A
	LEU	SER	AMBER	TRP	G
C	LEU	PRO	HIS	ARG	U
	LEU	PRO	HIS	ARG	C
	LEU	PRO	GLUN	ARG	A
	LEU	PRO	GLUN	ARG	G
A	ILEU	THR	ASPN	SER	U
	ILEU	THR	ASPN	SER	C
	ILEU	THR	LYS	ARG	A
	MET	THR	LYS	ARG	G
G	VAL	ALA	ASP	GLY	U
	VAL	ALA	ASP	GLY	C
	VAL	ALA	GLU	GLY	A
	VAL	ALA	GLU	GLY	G

Fig. 3. The genetic code consists of a one-to-one correspondence between each of the 64 best allocations (codons) for the 4 nucleotides (A,G,C, and U) and the 20 amino acids used by ribosomes for protein synthesis. There are definite assignments of specific amino acids to 61 out of the 64 codons. Methionine codon AUG initiates the protein synthesis and synthesis terminates at any chain-terminating (formerly called "nonsense") codons.

DNA Isolation, Fragmentation, and Purification

Isolation

DNA is readily isolated from lysed cells by phenol extraction followed by precipitation with alcohol. Various methods of cell lysis for DNA isolation have been developed which are suitable and

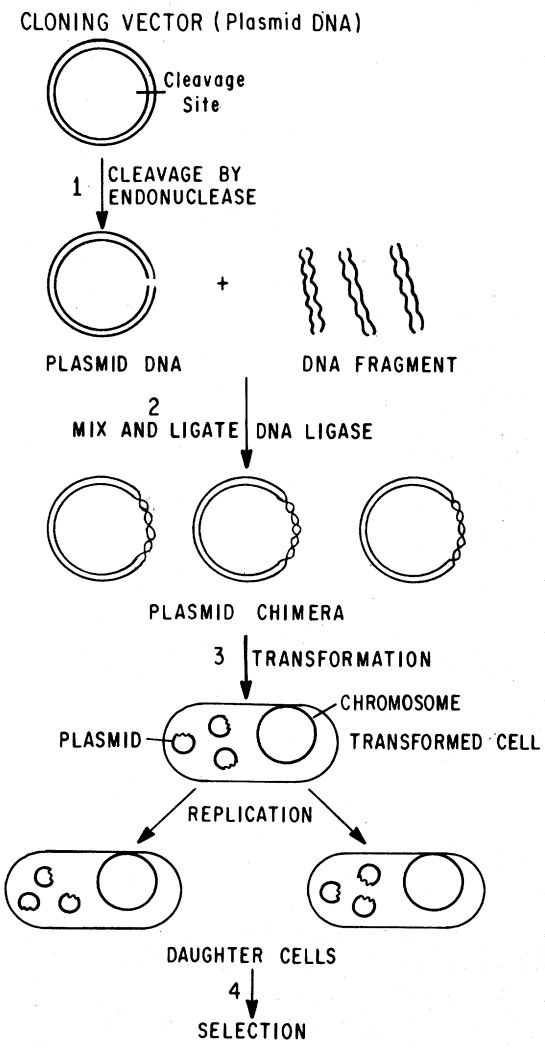

Fig. 4. Generalized scheme in gene cloning. (1) Isolation of DNA which bears the gene of interest by digestion with restriction endonuclease to generate the restriction fragment containing the gene of interest. The cloning vector is also digested with the same restriction endonuclease. (2) Fragments of the cleaved DNA containing the target gene are joined to the digested plasmid DNA by DNA ligase. (3) The ligated DNA (rDNA), each consisting of the entire plasmid, and the target gene are introduced into the host (*E. coli*) by transformation and DNA is replicated by virtue of the replication of the plasmid. (4) Selection of transformants containing the appropriate gene of interest.

Table 1. Cleavage Patterns of Restriction Endonucleases[a]

Blunt cleavage, Hae III	——GC ↓ CC—— ——CC ↑ GG——
Staggered cleavage, Eco RI	——G ↓ AATT C—— ——C TTAA ↑ G——

[a]Arrows represent recognition sequences and DNA cleavage sites by restriction endonuclease.

efficient for a variety of organisms (animals, plants, fungi, gram-positive and gram-negative bacteria, phages, and viruses) (Kolodner and Tewari, 1975; Schleif and Wensink, 1981; Crosa and Falkow, 1981; Perbal, 1984). The DNA fragments generated are usually purified further by techniques such as sucrose density gradients and dye-buoyant density gradient centrifugation (Schleif and Wensink, 1981; Crosa and Falkow, 1981; Perbal, 1984).

DNA Fragmentation and Purification

Physical shearing forces (vigorous mixing, drawing through a syringe needle or pipette, and ultrasonication) can be useful to generate short DNA fragments, some of which will contain the gene of interest. These procedures, however, produce random and irreproducible DNA fragments and are not generally suitable for the production of rDNA molecules. Instead, restriction endonucleases are usually used for the fragmentation of DNA (Nathans and Smith, 1975). More than 250 of these enzymes have been described, each having the ability to cleave double-stranded DNA at particular nucleotide sequences (Roberts, 1981). Table 1 illustrates the actual cleavage location in each strand relative to the recognition sequence for two common restriction endonucleases used in molecular cloning. Since the distribution of restriction sites within a DNA molecule is random, the average length of the fragments depends on the statistical occurrence of the particular restriction site in DNA. In most cases the fragments are about the size of a gene and therefore ideal for experiments involving gene cloning. These fragments can then be separated by agarose gel electrophoresis (Fig. 5) and the fragment containing the gene of interest is subsequently isolated and purified from the gel by electroelution (Perbal, 1984) or freezing and thawing techniques (Bhaduri et al., 1980; Goldenberg et al., 1981).

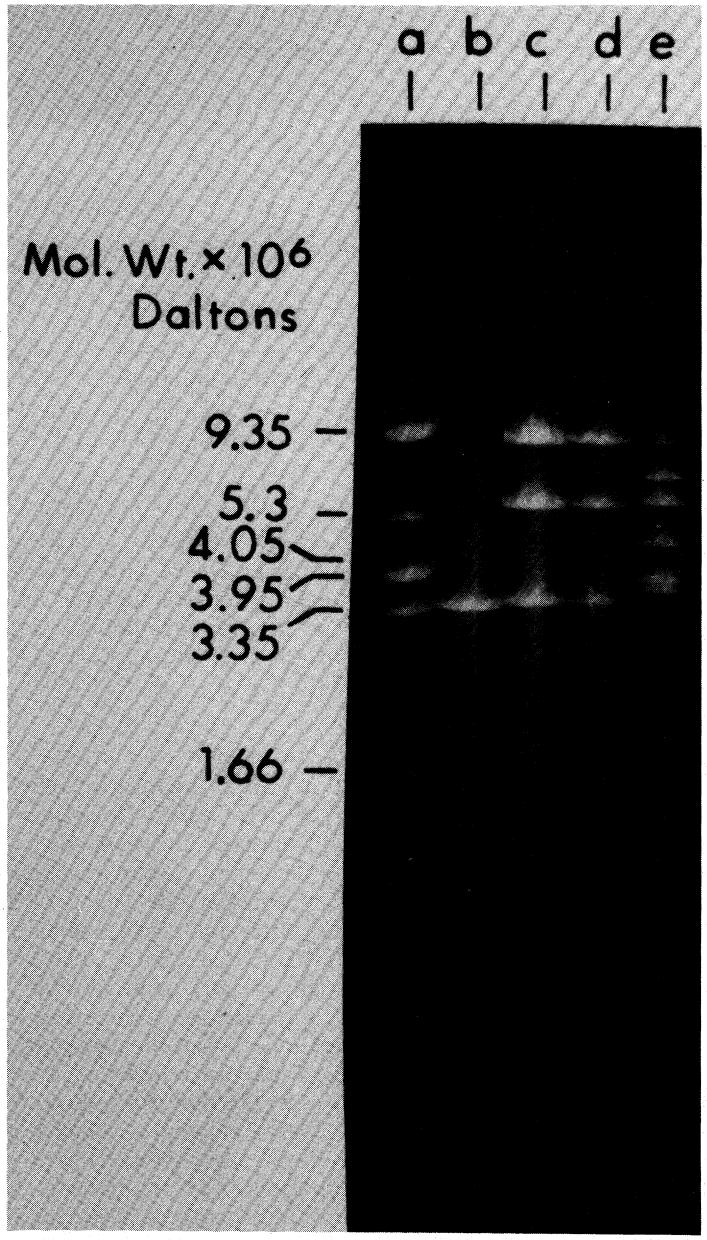

Fig. 5. Agarose gel electrophoresis of DNA fragments generated by restriction endonuclease. Electrophoresis was carried out at a constant 80 V for 4.5 hr in 0.7% agarose gel (Bhaduri et al., 1980).

Suitable Choice of Vector

Vectors are vehicles used to transfer and maintain DNA in foreign hosts. They are divided into two classes: plasmid and lambdoid phages. Since lambdoid phages can be expressed by only a few hosts, the following discussion will focus on plasmids. Plasmids are double-stranded, circular pieces of DNA found in bacteria which replicate independently of the bacterial chromosome. Genes for resistance to many antibiotics are often carried on plasmids (Fig. 6).

For ease of gene cloning, most researchers use small nonconjugative (low molecular weight) plasmids that posses readily identifiable markers (e.g., resistance to drugs or antibiotics). These plasmids must be capable of undergoing replication and amplification in an appropriate host. Additionally, plasmid DNA should posses a single restriction endonuclease cleavage site within resistance genes.

Several such vectors have been developed and specifically engineered for use in *Escherichia coli*. Two general types of plasmids should be selected for cloning: (1) those that are capable of very high copy numbers but restricted to a very small number of bacterial species, for example, pBR 322 in *E. coli* (Fig. 6) (Bolivar and Backman, 1979; Helinski, 1979), and (2) those that are derivatives of plasmids that can be maintained in a large number of bacterial species but which occur in relatively low copy numbers, for example, pRK 290 (Ditta et al., 1980). The high-copy number plasmid (1) is more useful for cloning any protein or peptide in a suitable host. The second type of plasmid (2) has potential application where modification of a "commercial microbe" is the objective; an example would be the cloning of useful genes (e.g., cellulase) into the nitrogen-fixing bacterium *Rhizobium* to obtain an organism that can convert wood (cellulose) into microbial protein. Table 2 lists some cloning vectors and their properties.

Gene Insertion into Vector and Introduction of Recombinant DNA into the Host

The gene (DNA fragment) to be cloned must be covalently joined to the cloning vector to generate an rDNA molecule. Such manipulations generally involve the specific cleavage of DNA preparation (passenger) with a restriction endonuclease. These DNA fragments are then mixed with a correspondingly cleaved vector DNA and joined end to end by DNA ligase followed by transfer (transformation or transfection) of circular rDNA molecules into a suitable host. Several strategies can be employed for ensuring that the desired DNA fragment is inserted in the correct position and orientation.

Cleavage of the DNA by a specific restriction endonuclease as indicated by arrows in Table 1 yields DNA fragments with either complementary single-stranded termini (i.e., Eco R1) causing

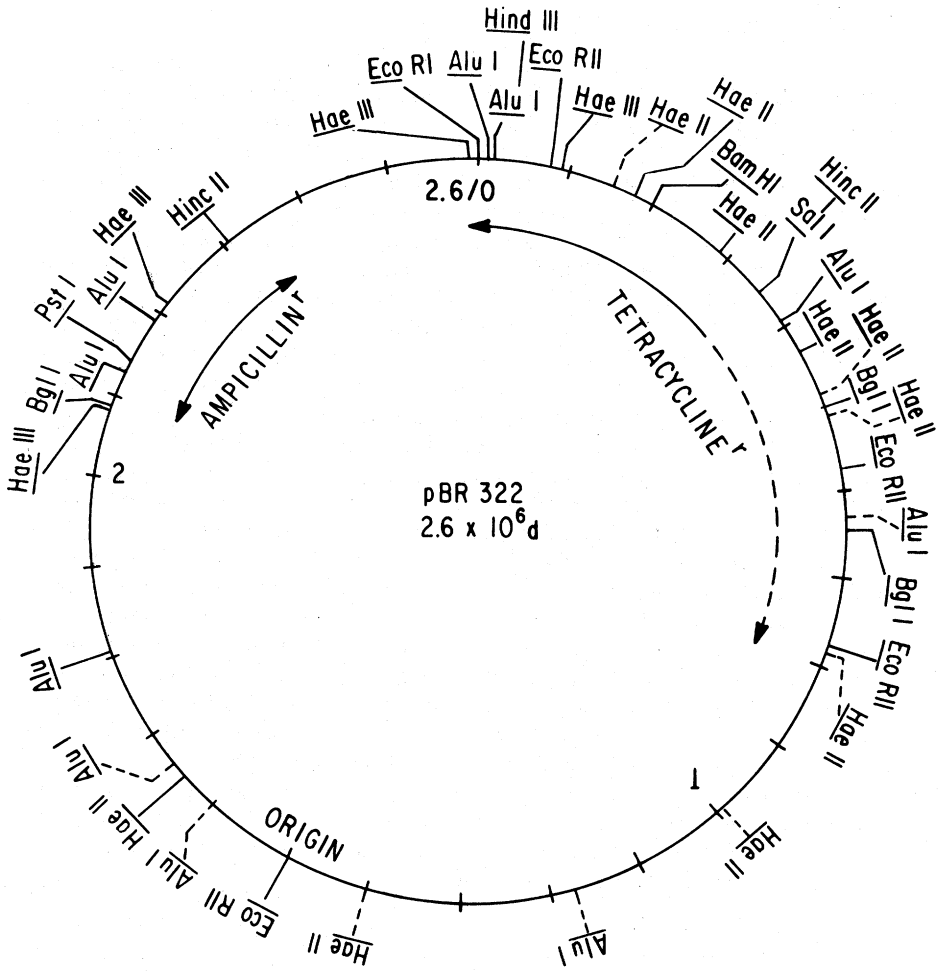

Fig. 6. Restriction map of pBR 322. Regions of the vector which confer resistance to the antibiotics ampicillin and tetracycline are indicated.

staggered or "sticky" cohesive ends. As is also shown in Table 1, cleavage can be opposite the phosphodiester bonds of the DNA duplex (i.e., Hae III), giving rise to blunt ends. The joining of passenger DNA (genes of interest) and vector DNA is easier in the case of the sticky ends. However, ligation can be achieved with T4 DNA ligase, which acts on both cohesive and blunt end DNA to yield rDNA.

Table 2. Plasmid Vectors for Gene Cloning

Vector	Host range	Comments
Gram-negative bacteria		
Col E1	*E. coli*	Can be amplified.
pBR 322	*E. coli*	The most common vector for general purpose use; can be amplified.
RSF 1010	*E. coli*	Some *Pseudomonas* spp. can be used as host.
pRK 290	*Klebsiella aerogenes*	Most gram-negative bacteria can be used as host.
RP1	*Pseudomonas aeruginosa*	Most gram-negative bacteria can be used as host.
Gram-positive bacteria		
pUB 110	*Bacillus*	Suitable for other *Bacillus* spp.
SCP–2	*Streptomyces*	Some *Streptomyces* spp. can also be used.
Fungi		
YEp; YRp	*Saccharomyces*	A broad-range vector system for yeast.
Plant cells		
Ti	*Agrobacterium tumefaciens*	At present only effective vector for cloning in plants.
Animal cells		
Defective SV40	Mammalian cell line	Control of transmittance is obtained in part by use of defective virus.

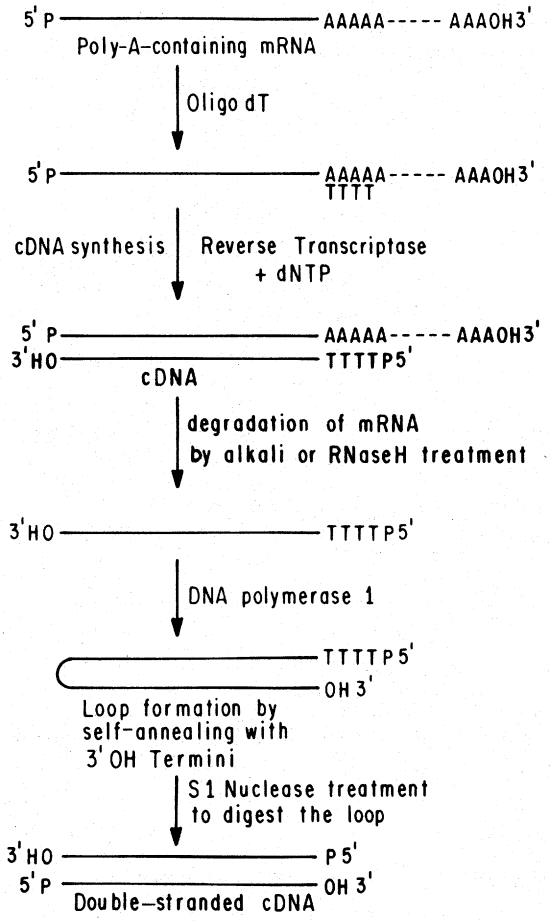

Fig. 7. Synthesis of double-stranded cDNA from poly-A containing mRNA. The schematic diagram is self-explanatory.

In certain cases problems arise. The restriction endonuclease cleavage site may be present within the structural gene of interest or the DNA fragment to be cloned may be extremely difficult to purify. In these cases different approaches have been employed (Craig and Hall, 1983; Harris, 1983). For example, a DNA fragment containing a gene (e.g., in the production of somatostatin and insulin) can be synthetically synthesized and then introduced into a plasmid. One effective approach to producing synthetic DNA is to use the messenger RNA (mRNA) for a desired cell product in conjunction

with enzyme reverse transcriptase plus four deoxynucleoside triphosphates. This enzyme uses the mRNA as a template to generate the complementary DNA (called cDNA). This method is preferred in cases where mRNA can be purified easily from a particular tissue. A schematic diagram is shown in Figure 7.

A second approach to gene cloning is chemical synthesis of DNA by a combination of organic and nucleic acid chemistry. Recent developments on the methods initiated by Khorana have made it possible to synthesize gene-sized fragments of DNA in several months, as with the synthesis of the insulin gene (Itakura et al., 1977; Crea et al., 1978). In this approach a DNA sequence is designed, based on the genetic code for a particular protein. In addition, cDNA and synthetic DNA do not contain the internal nontranslated sequence (introns) found in eukaryotic chromosomal DNA and are thus more suitable for expression of the gene in prokaryotes (Goldenberg et al., 1981). Synthetic DNA can also be cloned if a DNA contains the proper restriction site in the molecule.

When end-to-end ligation is not suitable to insert passenger DNA (particularly in the case of synthetic DNA) into the vector, homopolymeric tailing techniques (Deng and Wu, 1981) or the addition of synthetic linkers (Perbal, 1984) at the site of insertion can be employed. With homopolymeric tailing procedures, 15–20 nucleotides can be added to small amounts of DNA sequences. This process can be achieved with considerable accuracy over a reasonable time course (Fig. 8). Linkers containing the recognition and cleavage sites for specific restriction enzyme are joined to the passenger DNA by ligase and then inserted into the cloning vector as shown in Figure 9. A number of these linker molecules for different restriction enzymes are commercially available.

The introduction of rDNA into suitable hosts for the purpose of gene amplification or to obtain gene expression is carried out by the process of transformation. This involves inducing cells to incorporate rDNA. The cells which are capable of incorporating exogenous DNA without degradation are known as competent cells. Competence can often be induced by treatment with magnesium chloride and calcium chloride (Bhaduri et al., 1980; Goldenberg et al., 1981). Transformation is relatively inefficient, but if transformants (bacteria which have taken up plasmids) can be readily selected directly, the desired clone can usually be isolated.

Screening and Selection of Clones

Following transformation, the clone containing the desired gene must be identified. Clones constructed using the method described above contain plasmids with a range of inserts. The efficiency of the screening or selection method determines the success of a cloning

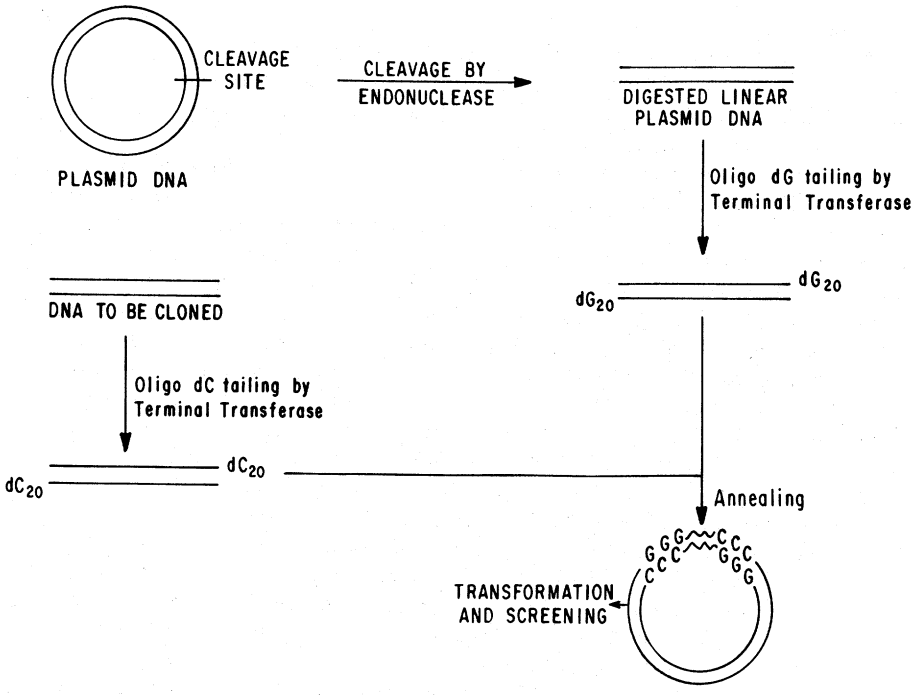

Fig. 8. Cloning of synthetic DNA in the plasmid vector by the homopolymeric dG:dC tailing technique.

project. The significance of this step becomes evident from the fact that some clones contain complete genes while others contain only fragments. The selection of the clones which contain the plasmid and produce the desired product is accomplished by a variety of methods (Fig. 10):

1. "Insertional inactivation" is the most widely used method, and it involves the insertion of a foreign DNA in the antibiotic resistance gene of plasmid, thereby causing a loss of that resistance. This method, however, only indicates that the plasmid contains foreign DNA.

2. Biological activity (an enzymatic or peptide hormone), the detection of expression of the gene product by its biological activity. For example, the clone containing specific enzyme may be detected by its specific action on the substrate. In the case of peptide hormones, the transformant containing the gene for the peptide hormone may be selected by its specific action in mouse assay.

3. Selective media, which support the growth of only those organisms that have taken up the desired gene. For example, transformants harboring the gene for histidine biosynthesis may be selected by growing a mixed population of transformants on media which does not contain histidine (Bhaduri et al., 1980).

4. Immunological detection of desired clones by radioisotope-labeled antibody specific for the product of the gene of interest (Craig and Hall, 1983).

5. Colony hybridization utilizes radioactive complementary nucleic acid probes (either DNA or RNA) which will bind with those clones containing the desired DNA sequence (Grunstein and Hogness, 1975; Doel et al., 1980).

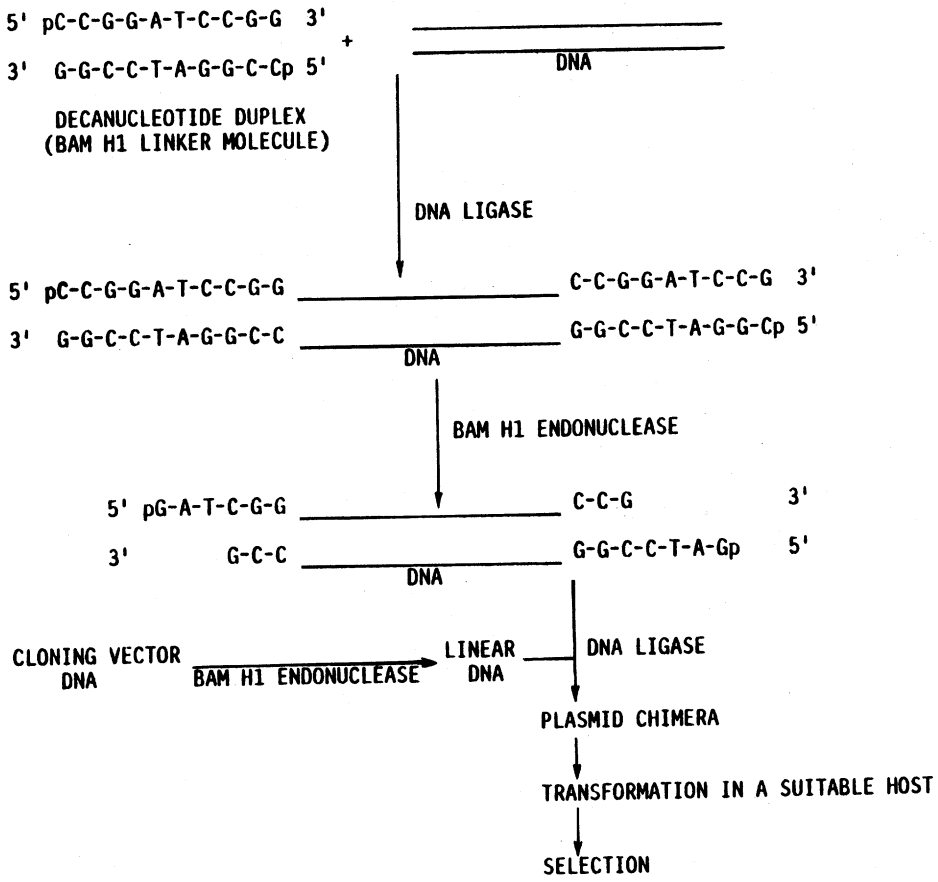

Fig. 9. Scheme for introducing cohesive ends at the termini of any duplex DNA molecule for molecular cloning.

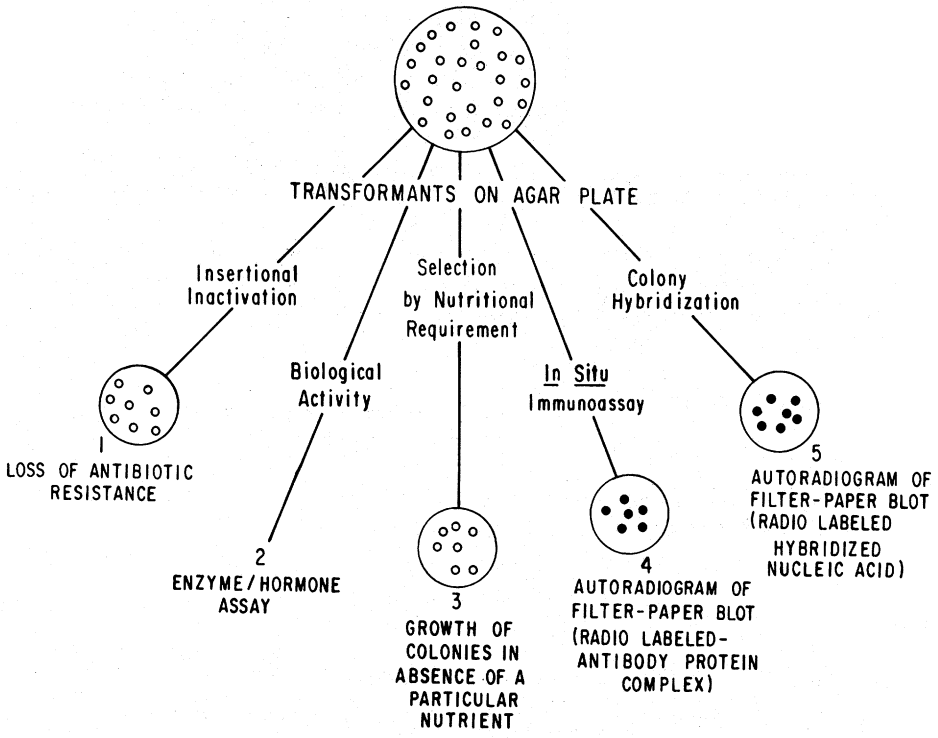

Fig. 10. The screening and selection of the desired clones include in vitro and in vivo assays and autoradiograms.

CURRENT STATUS OF RECOMBINANT DNA TECHNIQUES

A diverse range of vector systems has been developed for microbes, plants, and animals (see Table 2). These vector systems are available for their respective hosts and allow the use of rDNA techniques in other organisms (Struhl et al., 1979; Purchio and Fareed, 1979; Ream and Gordon, 1982). Useful vectors have also been developed for viruses (Old and Primrose, 1980). However, since the genetics of *E. coli* are so well defined, it is possible to achieve greater genetic manipulation within *E. coli* than with other organisms. The expression of unmodified cloned eukaryotic genes in bacteria is highly improbable, because prokaryotes appear to lack the enzymatic machinery needed to remove the intervening sequences (introns) that interrupt eukaryotic coding sequences (Goldenberg et al., 1981). Two different approaches can be used to solve this problem. One

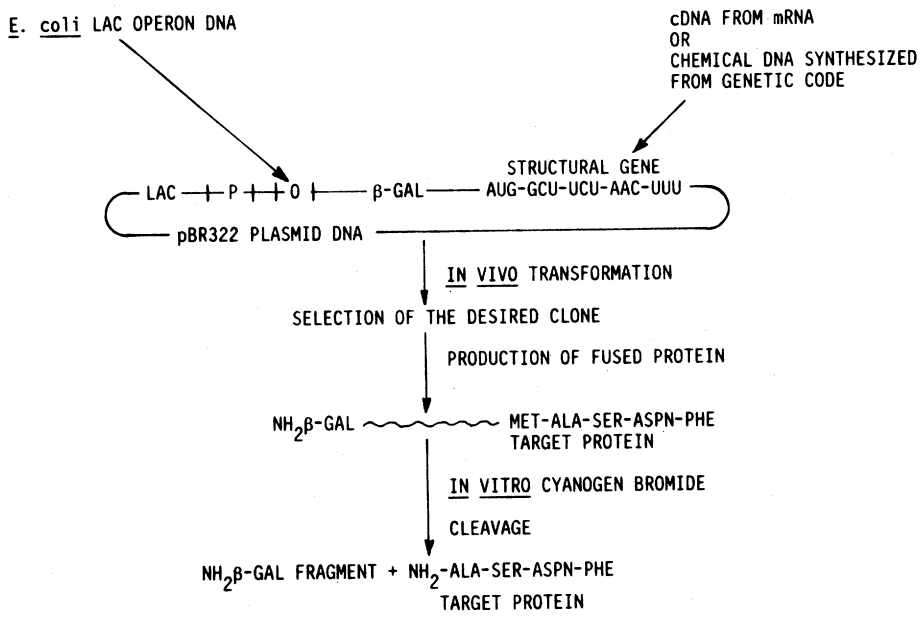

Fig. 11. Strategy for the expression of cDNA (gene) from mRNA or a chemically synthesized gene as a β-galactosidase fusion from the *lac* promoter. The active hormone can be cleaved from the hybrid protein by cyanogen bromide treatment.

is the insertion of cDNA and the other is the insertion of chemically synthesized DNA into a suitable vector. Both of these synthetic DNAs are "spliced copies" of the gene (Goldenberg et al., 1981) which can subsequently be introduced into expression-cloning vectors by standard techniques (Fig. 11).

APPLICATION OF RECOMBINANT DNA TECHNOLOGY IN THE FOOD INDUSTRY

The potential for the commercial exploitation of genetic engineering becomes more evident because of the development of the various techniques and approaches discussed previously. Although the food industry has historically been the oldest and most extensive user of biological processes for the production of its products, the use of applied genetics and modern biotechnology (rDNA technology) is currently quite limited. However, considerable research on the

genetic manipulation of microorganisms for the fermentative production of such foodstuffs as cheese and related fermented dairy products, alcoholic beverages, and acetic, lactic, and citric acids has been conducted with the objective of producing strains with superior activity, greater stability, or improved product yield. Effective use of rDNA technology could enhance progress in these areas. To date, a few processes have made use of rDNA technology.

Amino Acids

Amino acids are used to fortify the human and animal diet and as flavor enhancers. Currently these amino acids are obtained by extraction of biological materials or hydrolysis of protein. A high level of amino acid production in a microbial system could be achieved by the modification of the genes by rDNA technology. It has been estimated that the market for amino acid production will increase from $1.8 billion to $2.4 billion by the year 2000 as a result of rDNA techniques (Office of Technological Assessment, 1981).

Enzymes

Commercial use of enzymes by the food and detergent industries is valued at $160 million (Office of Technological Assessment, 1981). The common enzymes used in the industry are glucoamylase, glucose oxidase, exocellobiohydrolase-1, lipase, protease, isomerase, and invertase. These enzymes are isolated from fungi or bacteria which grow at slow rates and yield small amounts of enzyme. Other enzymes such as pepsin, rennin, and papain are of plant or animal tissue origin. Microbial production of these enzymes by genetic engineering could improve their yield at a significantly lower cost. Cloning of the genes of some of these enzymes (amylase, rennin) has been reported (White et al., 1984).

Single-Cell Protein

The use of genetic engineering in the production of single-cell protein has already been implemented (Windass et al., 1980; Gelfand, 1980). Genes from *E. coli*, which are more efficient in nitrogen assimilation, have been transferred into *Methylophilus methylotrophus*. The modified *Methylophilus* demonstrated an increased carbon conversion 4–7% over its parent. The production of single-cell protein from *Saccharomyces cerevisiae* is also in the process of development (Batt and Sinskey, 1984).

Starter Cultures

The genetic manipulation of starter culture by rDNA technology could enhance the efficiency and quality of the fermented food products. Studies (Kondo and McKay, 1982; Lee et al., 1982) at the molecular genetics level in dairy fermentation have already been initiated.

Polysaccharides

Polysaccharides are used as stabilizers and texturizers. Many are derived from plants. It would be cheaper if these were produced in bacteria through genetic manipulation by rDNA technology.

Sweeteners, Flavorants, and Pigments

Aspartame (dipeptide aspartylphenylalanine), used as a substitute sweetener, can be produced in bacteria by rDNA technology. Doel et al. (1980) chemically synthesized the gene, a dodecanucleotide specifying aspartylphenylalanine, and introduced it into a plasmid and expressed the gene as multiple repeating units of the aspartame dipeptide by cloning adjacent to a controllable tryptophan promoter. Proteolytic cleavage of this molecule by thermolysin yields only aspartame. The extremely sweet peptides monellin and thaumatins are derived from plants, and their production could be achieved by rDNA technology for commercial use. At present, the flavorants inosinic acid and guanylic acid are produced by extraction from yeast; it is possible to produce these compounds in bacteria by molecular cloning. The production of natural pigment carotenoids may also be enhanced by improvising the activity of their biosynthetic pathway by rDNA technology.

Other Potentially Important Applications of Recombinant DNA Technology to the Food Industry

1. Starch saccharification to glucose via microbially derived amylase
2. Microbial conversion of food processing wastes into edible products
3. Microbial removal of toxins or objectionable components from food to make safe, edible foods

FUTURE OF RECOMBINANT DNA TECHNOLOGY IN THE FOOD INDUSTRY

The discovery of rDNA technology and the current advances have brought about in a short time a new era in biological research. Although the techniques developed in rDNA technology are for general use and also applicable to diversified systems such as bacteria, fungi, plants, and animals, further innovative techniques will be required. The application of rDNA technology in the food industry depends not only on technical feasibilities but also on economic considerations. In the final analysis, it appears that genetic manipulation by rDNA technology in the food industry should be directed toward improved nutritive yield, productivity, and quality and constitutes the most promising outfall of genetics to the food industry within the next decade.

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GLOSSARY

Amplification refers to the production of additional copies of a chromosomal sequence, found as either intra- or extrachromosomal (plasmid) DNA.

Exons are the remaining segment of the primary transcript that are joined together to form final mRNA.

High-copy number plasmid refers to some plasmids under relaxed replication control, which means they continue replicating in large amounts (about 1000 genomes per cell) after bacteria stop dividing.

Introns are untranslated intervening sequences of DNA that are transcribed (primary transcripts) but excised within the primary transcript by splicing together the sequences (exon) on either side.

Linkers are short synthetic oligonucleotide duplex molecules containing the recognition sequence for some restriction enzymes.

Low-copy number plasmids are maintained in bacteria under stringent replication control, which means they replicate at a ratio of 1 or 2 plasmid DNAs for every host chromosome.

Splicing (RNA splicing) describes the excisions of introns and joining of exons in finished mRNA; thus introns are spliced out, while exons are spliced together.